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Ploszaj et al., Amino acids (Austria), 2000, 19 (2), p483-96.

Stefanelli et al. Biochemical journal (England) May 1, 2000, 347 Pt. 3, p875-80.

Sakagami et al. Anticancer Research (Greece), Jan-Feb. 2000, 20 (1A), p265-70.

Ray et al., American journal of physiology, Cell physiology (US), Mar. 2000, 278 (3), pC480-9.

Bock et al. Radiation research (US), Dec. 1999, 152 (6), p604-10.

Dai et al. Cancer research (US), Oct. 1, 1999, 59 (19), p4944-54.

Bratton et al. Jo. of biological chemistry (US), Oct. 1, 1999, 274 (40), p28113-20.

Palyi et al. Anti-cancer drugs (England), Jan 1999, 10 (10, p103-11.

Li et al. Am. journal of physiology , April 1999, 276 (4 Pt. 1), pC946-54.

Ray et al. Am. journal of physiology, Mar. 1999, 276 (3 Pt. 1) pC684-91.

Das et al. Oncology Research (US), 1997, 9 (11-12), p565-72.

Monti et al.. Life Sciences (England), 1998, 62 (9), p799-806.

Lin et al., Experimental cell research, (US), Nov. 25, 1997.

Tome et al. biochemical Journal (England) Dec. 15, 1997, 328 (Pt. 3), p847-54.

Hu et al., Biochemical journal (England), Nov. 15, 1997, 328 (Pt. 1), p307-16.

Tome et al. biological signals (Switzerland), May -Jun 1997, 6 (3), p150-6.

Taguchi et al., Cell biochemistry and function (England), Mar 2001, 19 (1), p19-26.

Camon et al. neurotoxicology (US), Fall 1994, 15 (3), p759-63.

Shinki et al., Gastroenterology (US), Jan 1991, 100 (1), p113-22.

Heston et al. Prostrate (US), 1982, 3 (4), p383-9

Stefanelli et al, biochemical journal (England), Apr. 1, 2001. 355 (pt. 1), p199-206.

Lopez et al., biocell: official journal of the sociedades latinoamericanas de microscopia electronica... et. al. 9Argentina), Dec. 1999, 23 (3), p223-8.

Schipper et al. seminars in cancer biology (US), feb. 2000, 10 (1), p55-68.

Nilsson et al., biochemical journal (England) Mar. 15, 2000, 346 Pt. 3, p699-704.

giuseppina monti m. et al., biochemical and biophysical research commun. (US), Apr. 13, 1999, 257 (2), p460-5.

ratasirayakorn et al, j. of periodontology feb. 1999, 70 (2), p179-84

27 stabellini et al., Experimental and molecular parhology (US), 1997, 64 (3), p147-55.

Sparapani et al, experimental neurology (US), nov. 1997, 148 (1), p157-66..

Dhalluin et al., carcinogenesis (Eng.), Nov. 1997, 18 (11), p2217-23.

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Direct Inhibitory Effect of Uremic Toxins and Polyamines on Proliferation of VERO Culture Cells

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The dialysate fluid of uremic patients exhibits, *in vitro*, an inhibitory effect on cell growth, owing to urea, guanidino compounds, and substances named middle molecules. The polyamines are compounds which exhibit high levels in biological fluids during either normal development or disease such as psoriasis, uremia, and tumors. Dialysate and middle molecules show toxicity and degeneration of the organotype cultures, whereas the free polyamines and nonrecirculated dialysate do not have any toxic effect. The aim of this study is to analyze the effects of polyamines, nonrecirculated dialysate, and middle molecules of uremic patients in periodic hemodialysis on cultured VERO (fibroblast-like cells) growth. These cells show an inhibition of growth in middle molecules or 2×10^{-4} *M* putrescine and a stimulation with nonrecirculated dialysate and 2×10^{-8} *M* putrescine. The effect is different because the cultures with middle molecules begin growth again after 24 hr, whereas in the presence of 2×10^{-4} *M* putrescine no further growth is observed. Cells maintained in middle molecules + 2×10^{-8} *M* putrescine show an irreversible degeneration, attesting a toxic effect due to the low molarities of putrescine. The electron microscopy shows alteration of cytoplasmic, mitochondrial, and nuclear membranes, but no chromatin fragmentation with either middle molecules or 2×10^{-4} *M* putrescine: this suggests that the cells do not die of apoptosis. In conclusion, during uremia the polyamines could cause toxic effects, even at low concentrations, on cells stressed by other toxic stimuli.

INTRODUCTION

In the blood of uremic patients there are certain substances (molecular weight range 350–5000 Da) involved in the clinical pictures of chronic renal failure, such as anemia and neuropathy (Man *et al.*, 1980). The dialysate fluid of uremic patients exhibits, *in vitro*, a cell growth inhibitory effect owing to urea, guanidino compounds (Nathan *et al.*, 1989), and substances named middle molecules (MM) (Stabellini *et al.*, 1985).

The polyamines (PAs) are organic cations of the human body (molecular weight range 150–350 Da) which exhibit high levels in biological fluids during normal development of red blood cells (Quemener *et al.*, 1991), liver regeneration (Marchesini *et al.*, 1992), or in diseases such as psoriasis, uremia, and tumors (Loser *et al.*, 1990). High urinary concentrations of putrescine reduce fractions, such as *N*-acetylputrescine, and predict acute rejection in heart transplanted patients (Carrier *et al.*, 1990), whereas prediction of PA response to chemotherapy and tumor size in cancer patients (Lawton *et al.*, 1990) is uncertain.

In our previous reviews (Stabellini *et al.*, 1985) we have demonstrated the presence of PAs in dialysate of uremic patients and in the very chromatographic peak where MM separate from other substances in the dialysate.

In vitro high concentrations of PAs exhibit an inhibitory effect on cell growth (Stabellini *et al.*, 1989), while the plasma concentrations of uremic patients have no effect (Stabellini *et al.*, 1993). Total recirculated dialysate containing MM and traces of PAs (Stabellini *et al.*, 1982) shows toxicity with degeneration of the organotype cultures, whereas the free PAs and the nonrecirculated dialysate do not have any toxic effect

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(Stabellini *et al.*, 1985). PAs interact with DNA and RNA, showing a significant role in cell proliferation and differentiation (Heby, 1981; Stabellini *et al.*, 1995). PAs stabilize cell-free DNA to enzymatic degradation (Brune *et al.*, 1991) and have a protective effect on DNA fragmentation and the apoptosis process (Brune *et al.*, 1991).

The aim of this study is to analyze the effects that PAs, nonrecirculated dialysate, and recirculated dialysate fluid of terminal uremic patients in periodic hemodialysis have on the growth of cultured fibroblast-like cells (VERO).

MATERIALS AND METHODS

Cell culture. Fibroblast-like cells (VERO) were grown in Dulbecco's minimum essential medium (DMEM) (GIBCO BRL; UK) supplemented with 10% fetal calf serum (FCS) at a density of 250×10^3/ml. Cells were incubated in the presence of 8, 12, or 15 mg/ml MM or 2×10^{-4}, 2×10^{-8} *M* putrescine (PUT) (Sigma Chemical Co., St. Louis, MO). At the end of regular recirculated hemodialysis, dialysates (1000 ml each) were obtained from 10 patients with renal failure, chosen at random. The procedure of regular recirculated hemodialysis was carried out with a cuprophane filter and 40 liters of bicarbonated bath (Ca^{2+} 4.0 mEq/liter, Mg^{2+} 1.0 mEq/liter, K^+ 1.0 mEq/liter, Cl^- 100 mEq/liter, Na^+ 138 mEq/liter, acetate 10 mEq/liter) for 3 hr, three times weekly. Dialysate samples were lyophilized and pooled; 1 mg of lyophilized power was eluted with 1 ml 0.1 *M* Tris–HCl, pH 8.6, and MM were obtained by chromatography performed at 2°C using Sephadex G 15 (Pharmacia, Sweden) according to Furst *et al.* (1976). The tube solutions corresponding to 10 chromatographic II peaks (representing the peak of MM standardized with vitamin B12) were pooled, dialyzed, lyophilized, and stored at −20°C. High-performance liquid chromatography (HPLC) analysis revealed traces of PAs, in accordance with previous research (Stabellini *et al.*, 1982). In the presence of PAs and MM, [^{3}H]thymidine was added during exponential growth. At the same exponential phase, cells were counted after 24, 48, and 72 hr, prepared for analysis of thymidine incorporation and DNA gel electrophoresis, and fixed for light and transmission electron microscopy.

[^{3}H]Thymidine incorporation. Control and treated cells were pulsed with 1.0 μCi [^{3}H]thymidine (20 Ci/mmol NEN, Bad Homburg, Germany) during the 24, 48, and 72 hr of the culture period. Cells were solubilized in 0.5 *M* NaOH; an aliquot was precipitated with 10% TCA (30 min at 4°C) filtered on glass microfiber filters (GF/C Whatman, Maidstone, England) and washed with cold 5% TCA (TCA insoluble fraction). The filters were dried and counted in 10 ml of scintillation fluid (Pico Fluor 40, Packard, Downers Grove, IL) in a Packard 2425 scintillation counter. Total cell radioactivity (uptake) was measured in separate aliquots of solubilized cells counted in 10 ml of scintillation fluid. The results are expressed as dpm/cell proteins.

DNA gel electrophoresis. DNA gel electrophoresis was routinely extracted and purified as reported by Boe *et al.* (1991). Then it was run on a 0.8% agarose gel, as described by Falcieri *et al.* (1993). Molecular weight markers were from Boehringer–Mannheim (Germany).

Electron microscopy. Cultures were removed after 24, 48, and 72 hr using 2 ml trypsin 0.1% in Hearle without Ca^{2+} and Mg^{2+} and centrifuged at 50*g* for 10 min. The pellet was fixed in glutaraldehyde (2.5% in 0.1 *M* phosphate buffer) for 1 hr at 4°C, postfixed in osmium tetroxide (2% in 0.1 *M* phosphate buffer) for 30 min, and then embedded in araldite. Thin sections were cut on an ultramicrotome with glass knives, stained with uranyl acetate and lead citrate, and examined with an Hitachi 800 transmission electron microscope. For light microscopy studies, the monolayer cells were fixed in acetone for

TABLE I

Percentage Values of VERO Cell Proliferation in the Presence of Middle Molecules (MM), Putrescine (PUT), and Nonrecirculated Dialysate Bath (BD)

	Time of culture (hr)		
	24	48	72
8 mg/ml MM	37.8 ± 5.9	13.8 ± 6.8	-3.4 ± 9.3
12 mg/ml MM	69.4 ± 10.6	-50.6 ± 9.0	-44.0 ± 9.6
15 mg/ml MM	79.3 ± 10.3	-75.0 ± 7.7	-66.0 ± 9.3
15 mg/ml BD	+51.6 ± 12.0	+30.2 ± 9.3	+15.0 ± 8.7
2×10^{-8} *M* PUT	+77.7 ± 12.1	+50.0 ± 6.5	+45.0 ± 11.2
2×10^{-4} *M* PUT	−33.0 ± 8.4	−70.0 ± 9.0	Dead
2×10^{-8} *M* PUT + 12 mg/ml MM	−55.5 ± 12.3	−85.5 ± 12.1	Dead
2×10^{-4} *M* PUT + 12 mg/ml MM	−56.0 ± 9.1	−75.0 ± 10.5	Dead
2×10^{-8} *M* PUT + 15 mg/ml MM	67.0 ± 5.9	-80.2 ± 12.4	Dead
2×10^{-4} *M* PUT + 15 mg/ml MM	66.6 ± 5.9	70.1 ± 6.8	Dead

Note. Values are means ± SD in 10 duplicated experiments.

10 min, stained with hematoxylin and eosin, mounted, and observed with a Leitz microscope.

RESULTS

Cell culture. Table I shows that VERO cultures with MM have 37.8% value of growth inhibition at 8 mg/ml concentration, 69.4% at 12 mg/ml, and 79.3% at 15 mg/ml after 24 hr of culture. After 48 hr incubation, inhibition is reduced to 13.8% at 8 mg/ml, 50.6% at 12 mg/ml, and 75.0% at 15 mg/ml. The trend is maintained also after 72 hr. Nonrecirculated dialysis fluid cultures show a 51.6% increase of proliferation after 24 hr incubation, 30.2% after 48 hr, and 15% after 72 hr. Cultures with 2×10^{-8} *M* PUT have a greater proliferation (+77.7%), while cultures with 2×10^{-4} *M* PUT display an inhibition (−33.3%) after 24 hr incubation compared to control. The effect of 2×10^{-8} *M* concentration decreases after 48 hr incubation, while the inhibitory effect of 2×10^{-4} *M* concentration increases, with the death of all cells at 72 hr incubation. After 24 hr, inhibition of proliferation is present both in MM + 2×10^{-8} *M* PUT cultures and in MM + 2×10^{-4} *M* PUT cultures: all cultures die after 72 hr incubation.

Cultures previously incubated with MM (8, 12, and 15 mg/ml) for 24 hr and then incubated with DMEM + FCS alone for 24, 48, or 72 hr show a trend of progressive improvement in proliferation of the VERO cells (Table II).

TABLE II

Percentage Values of VERO Proliferation after 24 hr Culture with Medium Containing Middle Molecules (8, 12, and 15 mg/ml) Changed with DMEM + 10% Fetal Calf Serum Alone and Evaluated after 24, 48, and 72 hr

	Time of culture (hr)		
	24	48	72
8 mg/ml MM	−43.0 ± 7.6	−20.0 ± 7.0	−13.0 ± 6.1
12 mg/ml MM	−49.0 ± 5.8	−20.0 ± 6.4	−16.0 ± 5.6
15 mg/ml MM	54.0 ± 6.2	36.0 ± 5.9	−26.0 ± 7.0

Note. Values are means ± SD in 10 duplicated experiments. MM, middle molecules.

TABLE III
Cell Proliferation of Fresh VERO Cells with Additional Medium Containing MM and Previously Utilized for 24 hr

	Time of culture (hr)		
	24	48	72
8 mg/ml MM	−5.5 ± 2.8	+10.1 ± 4.8	+35.2 ± 7.7
12 mg/ml MM	−22.5 ± 5.3	−2.0 ± 1.5	+19.5 ± 5.8
15 mg/ml MM	−56.5 ± 6.9	−30.6 ± 7.5	−10.1 ± 5.5

Note. Values are means ± SD in 10 duplicated experiments. MM, middle molecules.

The medium containing MM, used for 24 hr, is still able to inhibit the growth of fresh cultures (Table III). In fact there is a 5.5% inhibition with 8 mg/ml, 22.5% with 12 mg/ml, and 56.5% with 15 mg/ml after 24 hr incubation. After 48 hr the cultures with 8 mg/ml increase the number of cells + 10.1%, whereas at 12 and 15 mg/ml of MM the inhibition diminishes compared to that at 24 hr (−2.0 and −30.6, respectively). The trend is maintained at 72 hr of culture.

[^{3}H]Thymidine incorporation. Table IV shows that after 24 hr incubation, [^{3}H]thymidine incorporation increases ($P < 0.01$) in the presence of 2×10^{-8} *M* PUT and nonrecirculated dialysate bath (BD), while it decreases ($P < 0.01$) in the presence of MM and 2×10^{-4} *M* PUT. The same trend is maintained after 48 and 72 hr incubation.

DNA gel electrophoresis. After 24, 48, and 72 hr incubation with 8, 12, and 15 mg/ml MM and 2×10^{4} *M* or 2×10^{-8} *M* PUT, DNA gel electrophoresis does not show any difference with respect to the control (Fig. 1).

Ultrastructural data. In control cultures (Fig. 2) VERO cells show an irregular cell surface, due to the presence of numerous extensions that appear either as villi of different sizes or as real plasmic pseudopodi-like extroflections. The cytoplasm is rich in vacuoles surrounded by membrane, filled with dense bodies that sometimes appear as overlapping lamellae. Free ribosomes are numerous, scattered throughout the cytoplasm or clustered in rosettes. Tubuli of rough endoplasmic reticulum are scarcely evident. Mytochondria show characteristic double membranes and numerous transversal crests. The voluminous nuclei have regular rims, with occasional shallow introflections. These nuclei are delimited by double membranes with interposed perinuclear cisterna. On the inner membrane numerous ribosomes are more densely present in a single layer. Chromatin is finely dispersed, and the nucleolus is evident in both the filamentous and the globular parts.

TABLE IV
[^{3}H]Thymidine Incorporation during 24 hr of Culture with Putrescine (PUT), Nonrecirculated Dialysate Bath (BD), and Middle Molecules (MM)

	Time of culture (hr)		
	24	48	72
2×10^{-4} *M* PUT	186.7 ± 25.8*	50.8 ± 31.5*	Dead
2×10^{-8} *M* PUT	1355.5 ± 199.7*	1720.8 ± 231.9*	1802.6 ± 332.2*
12 mg/ml MM	100.3 ± 30.6*	119.4 ± 30.7*	125.4 ± 38.6*
15 mg/ml MM	76.7 ± 20.7*	73.6 ± 22.2*	84.4 ± 23.4*
15 mg/ml BD	1679.5 ± 197.1*	2183.2 ± 255.8*	2435.1 ± 833.2*

Note. Values are means ± SD in five duplicated experiments.
* $P < 0.01$ compared to controls.

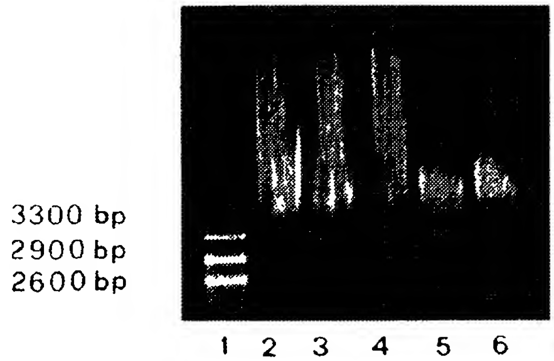

FIG. 1. DNA gel electrophoresis of VERO cells maintained for 24 hr in DMEM + 12 mg/ml MM (lane 2), 15 mg/ml BD (lane 3), 2×10^{-4} *M* PUT (lane 4), 2×10^{-8} *M* PUT (lane 5), and controls (lane 6). Lane 1, Molecular weight.

After 24 hr culture in medium containing MM, VERO cells show evident signs of suffering, such as numerous empty cytoplasmic vacuoles and lost cytoplasmic organelles. Both nuclei and nucleoli show gross granulation; the nuclear rim is delimited by a scarcely evident membrane without perinuclear cisterna (Fig. 3).

In the presence of 2×10^{-4} *M* PUT, the ultrastructural features of VERO cells are exactly the same as those of cells cultured in medium containing MM, as regards the morphologic characteristics of the nuclei, numerous vacuoli, and scarce cellular organelles (Fig. 4).

VERO cells cultured in medium containing 2×10^{-8} *M* PUT present the same ultrastructural features as controls (Fig. 5); the cytoplasm is rich in mytochondria and free diffuse ribosomes. The plasma membrane is complicated by the presence of microvilli.

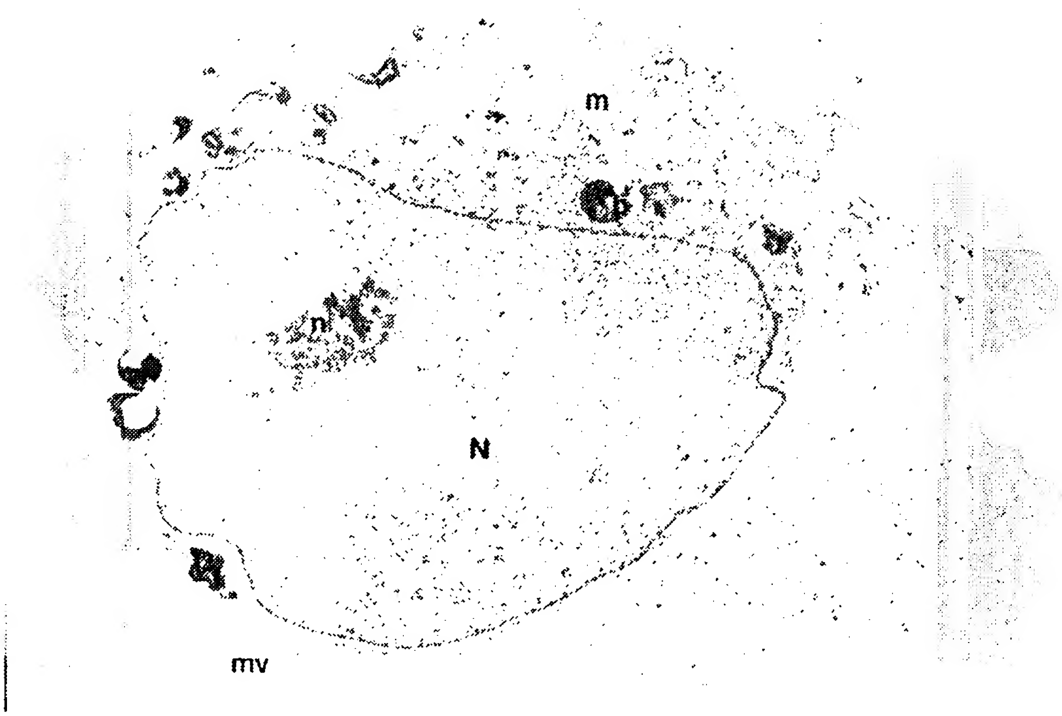

FIG. 2. Electron micrograph of control VERO cells maintained for 24 hr in culture. N, nucleus, n, nucleolus; mv, microvilli, m, mitochondrium, b, dense bodies. Original magnification, ×15,000.

Rough endoplasmic reticulum tubuli are scarcely evident, while the numerous vacuoli contain dense lamellae. The voluminous nuclei are delimited by a typical nuclear envelope; chromatin is evenly dispersed as fine granules. Nucleoli are clearly evident in both the filamentous and the globular parts.

DISCUSSION

Our study of the PUT, BD, and MM effects on the proliferation of VERO cultures shows an inhibitory effect of either MM or 2×10^{-4} *M* PUT and a stimulating effect of either BD or 2×10^{-8} *M* PUT. It is well known that acetate present in BD has a stimulating effect (Shutches *et al.*, 1981) and that 2×10^{-8} *M* PUT has a stimulating effect on cellular activity, while 2×10^{-4} *M* PUT and MM have an inhibitory effect on cell proliferation (Stabellini *et al.*, 1989). The stimulating effect of nonrecirculated BD shows that the inhibition of proliferation is not dependent on variations of pH and concentrations of substances present in the BD added to culture medium, as already demonstrated by Weislander *et al.* (1991).

Our data confirm that the inhibition of proliferation is due to substances present also in

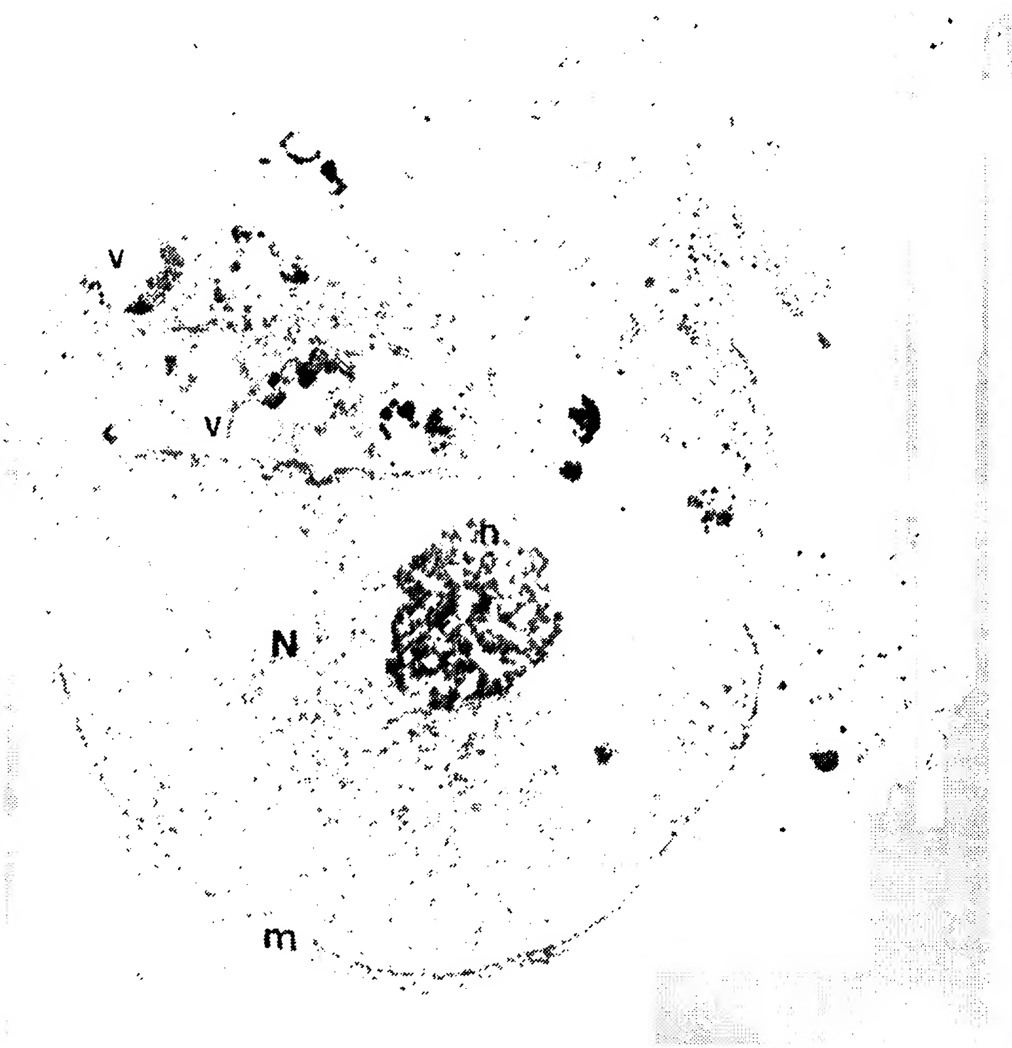

FIG. 3. Electron micrograph of 24-hr-cultured VERO cells in the presence of middle molecules. N, nucleus; n, nucleolus; m, mitochondrium; v, vacuole. Original magnification, ×11,000.

FIG. 4. Electron micrograph of 24-hr-cultured VERO cells in the presence of 2×10^{-4} *M* putrescine. N, nucleus; m, mitochondrium; v, vacuole. Original magnification, ×11,000.

recirculated dialysis fluid and then withdrawn from patients' blood during dialysis, because the PAs do not show toxicity at values of concentrations present in recirculated BD (Stabellini *et al.*, 1993). However, the toxic effect is different: in fact, in the presence of uremic toxins, cultures resume proliferation after 24 hr incubation, while those in the

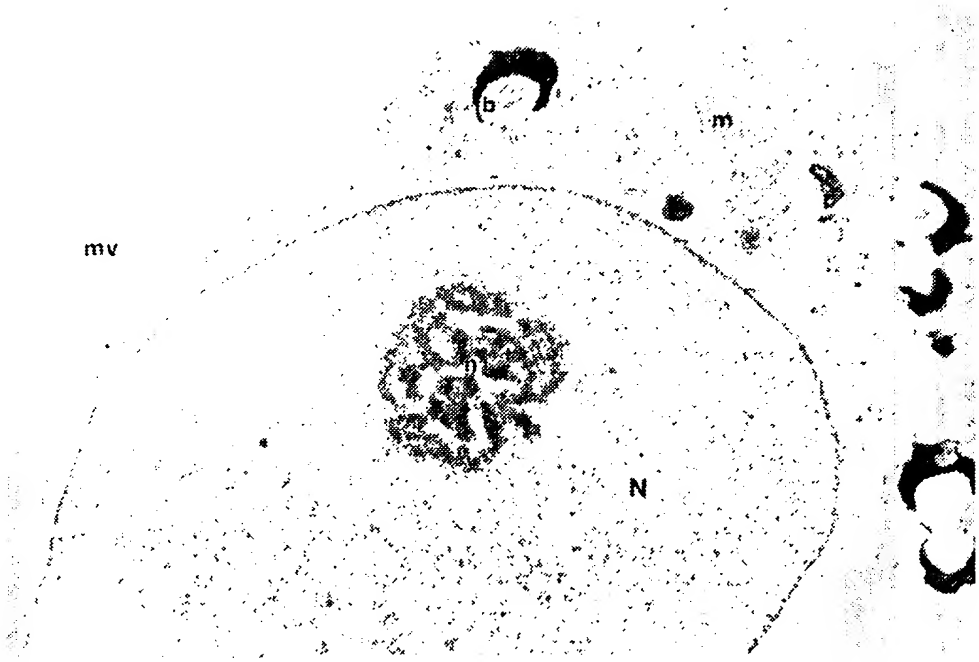

FIG. 5. Electron micrograph of 24-hr-cultured VERO cells in the presence of middle molecules and 2×10^{-8} *M* putrescine. N, nucleus; n, nucleolus; mv, microvilli; m, mitochondrium; b, dense bodies. Original magnification, ×20,000.

presence of PUT die. This difference is not due to the disappearance of the MM toxic effect, because we have noticed that fresh cultures added with medium incubated for 24 hr suffer the same inhibitory effect; rather, it seems to be an adaptation of the VERO cells.

Electron microscopy shows that MM or 2×10^{-4} M PUT produces alteration of the nuclear membrane and mitochondria, but the absence of chromatin fragmentation could indicate that the cells do not die of apoptosis. Moreover, the nonhomogeneity of the VERO population may partially explain the different effects of MM and PUT on proliferation. It is interesting to note that cells incubated in the presence of MM + 2×10^{-8} M PUT come up against irreversible degeneration, thereby showing a toxic effect of this PA on all possible present cells. However, our results could be due not only to the toxic effect of PUT but also to the stimulation of suffering cells by now unable to respond to action of PA. In conclusion, during uremia PAs may cause deadly effects, even at low concentrations, on cells stressed by other harmful stimuli.

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REFERENCES

Boe, R., Gjertsen, B. T., Vintermyr, O. K., Houge, G., Lanotte, M., and Doskeland, S. O. (1991). The protein phosphatase inhibitor okadaic acid induces morphological changes typical of apoptosis in mammalian cells. *Exp. Cell Res.* **195**, 237–246.

Brune, B., Hartzell, P., Nicotera, P., and Orrenius, S. (1991). Spermine prevents endonuclease activation and apoptosis in thymocytes. *Exp. Cell Res.* **195**, 323–329.

Carrier, M., Russell, D. H., Cork, R. C., Wild, J., Emery, R. W., and Copeland, J. G. (1990). Analysis of risk factors for acute allograft rejection after heart transplantation. *J. Heart Transplant.* **9**, 372–375.

Falcieri, E., Martelli, A. M., Bareggi, R., Cataldi, A., and Cocco, L. (1993). The protein kinase inhibitor staurosporine induces morphological changes typical of apoptosis in Molt-4 cells without concomitant DNA fragmentation. *Histochemistry* **193**, 19–25.

Furst, P., Zimmerman, L., and Bergström, J. (1976). Determination of endogenous middle molecules in normal and uremic body fluids. *Clin. Nephrol.* **5**, 178–188.

Heby, O. (1981). Role of polyamines in the control of cell proliferation and differentiation. *Differentiation* **19**, 1–20.

Loser, C., Folsch, U. R., Paprotny, C., and Creutzfeldt, W. (1990). Polyamines in colorectal cancer. *Cancer* **65**, 958–966.

Lawton, F. G., Griffin, M., Slack, J. A., and Blackledge, G. (1990). Predicting response to chemotherapy for patients with epithelial ovarian cancer using urinary polyamine excretion patterns. *Br. J. Cancer* **62**, 692–694.

Man, N. K., Cueille, G., Zingraff, J., Boudet, J., Sausse, A., and Funk-Brentano, J. L. (1980). Uremic toxins in the middle molecular weight range. *Artificial Organs* **4**, 116–120.

Marchesini, G., Checchia, G. A., Stefanelli, C., Bianchi, G., Fabbri, A., Zoli, M., Caldarera, C. M. (1992). Polyamine plasma levels and liver regeneration following partial hepatic resection in man. *J. Hepatol.* **16**, 159–164.

Nathan, I., Hensel, R., Dvilansky, A., and Shainkin-Kestenbaum, R. (1989). Effect of uremic toxins-guanidino compounds and creatinine-on proliferation on HL60 and K562 cell lines. *Nephron* **52**, 251–252.

Quemener, V., Martin, C., Havouis, R., Moulinoux, J. P. (1991). Red blood cell polyamine evolution during normal growth in mouse. *In Vivo* **5**, 91–94.

Shutches, C. L., Sigler, M. H., Teehan, B. P., Cooper, J. H., and Reichard, G. A. (1981). Contribution of dialysate acetate to energy metabolism. Metabolic implication. *Kidney Int.* **23**, 57–59.

Stabellini, G., Spisani, S., Fiocchi, O., Stabellini, N., Carletti, R., Altobelli, A., Farinelli, A. (1982). Tossine uremiche mediomolecolari e poliamine endogene. Comportamento della chemotassi dei polimorfonucleati rispetto ai picchi cromatografici di dialisato e poliamine standard. *Boll. Soc. It. Sper.* **LVIII**, 1418–1424.

Stabellini, G., Fiocchi, O., Stabellini, N., Malacarne, F., Farinelli, R., Spisani, S., and Farinelli, A. (1985). Valutazione dell'attività delle tossine uremiche e delle poliammine su colture organotipiche di embrione di pollo di 7 giorni di sviluppo. *Boll. Soc. It. Biol. Sper.* **LXI**, 387–393.

Stabellini, G., Fiocchi, O., Evangelisti, R., Pagliarini, A., Becchetti, E., and Caruso, A. (1989). Effects of

spermine and spermidine on glycosaminoglycan metabolism in primary cultures of chick embryo fibroblasts. *Med. Sci. Res.* **17,** 915–916.

Stabellini, G., Fiocchi, O., Caruso, A., Bedani, P. L., De Mattei, M., Gilli, P., Gavioli, R., and Spisani, S. (1993). Effect of polyamines, acetylderivatives and uraemic toxins on neutrophil functions. *Med. Sci. Res.* **21,** 255–257.

Stabellini, G., Rapino, M., Di Primio, R., and Trubiani, O. (1995). Polyamines and terminal deoxynucleotidyl transferase expression in KM 3 pre-B cell line during phorbol ester induced differentiation. *Cell Biol. Int.* **19,** 821–825.

Weislander, A. P., Nordin, M. K., Kjellstrand, P. T. T., and Boberg, U. C. (1991). Toxicity of peritoneal dialysis fluids on cultured fibroblasts. *Kidney Int.* **40,** 77–79.